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IDENTIFICATION, SYNTHESIS, AND BIOACTIVITY OF A MALE-PRODUCED AGGREGATION PHEROMONE IN ASSASSIN BUG, *Pristhesancus Plagipennis* (HEMIPTERA: REDUVIIDAE)

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Abstract—*Pristhesancus plagipennis*, a large Australian assassin bug, possesses three pairs of dorsal abdominal glands (DAGs). In the male, the anterior and posterior glands are hypertrophied and secrete an attractant pheromone. Gas chromatography–mass spectrometry (GC-MS) analyses of male DAG extracts and airborne volatiles emitted from calling males showed the pheromone signature to be dominated by a novel component. Subsequent chemical manipulations, GC-MS, and chiral-column analyses established its identity as (*Z*)-3-hexenyl (*R*)-2-hydroxy-3-methylbutyrate. Minor components included 3-methylbutanol, 2-phenylethanol, (*Z*)-3-hexenol, decanal, (*E*)-2-hexenoic acid, and three minor hexenyl esters. Bioactivity studies using laboratory olfactometers and outdoor flight cages demonstrated attraction by female *P. plagipennis* to calling males, heptane extracts of male posterior DAGs and a synthetic formulation of the (*Z*)*R* enantiomer of the major ester, alone or in combination with other components of male anterior and posterior DAGs. Males were also attracted to the major ester. The racemate and *S* enantiomer of the ester were not attractive. Contamination of the (*Z*)*R* enantiomer with 30–60% of the *E* isomer also made the compound nonattractive. This is the

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first report of an aggregation pheromone in the Reduviidae. The prospects for pheromonal manipulation of *P. plagipennis* populations to enhance the value of this predator in horticultural ecosystems, are discussed.

Key Words—*Pristhesancus plagipennis*, assassin bug, Hemiptera, Reduviidae, dorsal abdominal glands, (*Z*)-3-hexenyl (*R*)-2-hydroxy-3-methylbutyrate, aggregation pheromone.

INTRODUCTION

The chemical ecology of assassin bugs (Reduviidae), the largest family of predaceous land bugs (Schaefer, 1988), has been little studied. Aldrich (1988) suggested that the sedentary mode of capturing prey and use of the rostrum for stinging defense may have contributed to reduction of the metathoracic gland and nymphal dorsal abdominal glands (DAGs). Most reduviids possess two pairs of unique glands: Brindley's glands situated laterally under the first abdominal tergite and ventral glands at the junction of the thorax and abdomen (Staddon, 1979). Secretions from Brindley's glands in hematophagous reduviids (Triatominae) consist primarily of isobutyric acid (Blum, 1981). Clear functions for reduviid exocrine glands have not been established.

Pristhesancus plagipennis Walker is a large (2–2.5 cm), common Australian reduviid that is part of the natural enemy complex operating against the spined citrus bug, *Biprorulus bibax* Breddin (Pentatomidae) in Queensland helping to prevent economic damage (Summerville, 1931; Murray, 1987; James, 1992). Dissection of adult *P. plagipennis* revealed the presence of three well-developed paired DAGs, with the anterior and posterior pair being much larger in males than females. In addition, males were observed to adopt a characteristic calling posture accompanied by release of volatiles (Aldrich, 1991). Recently, *B. bibax* has become a serious pest of citrus in southern Australia (James, 1989) where *P. plagipennis* does not occur naturally. Integrated management of *B. bibax* in southern areas is focused on utilizing and strengthening the natural enemy complex, by introducing parasitoids and predators from elsewhere in Australia (James, 1993). Initial introduction of *P. plagipennis* to the Murrumbidgee Irrigation Area in southern New South Wales were made in 1991–1992, but were hampered by difficulties in collecting and rearing sufficient numbers of bugs.

Associated studies on pheromones of *B. bibax* (James and Warren, 1989; James et al., 1990) led us to examine the exocrine gland morphology of *P. plagipennis*. This paper verifies the existence of sexually dimorphic DAGs in *P. plagipennis* and describes the identification, synthesis, and bioactivity of a male-produced aggregation pheromone emanating from these glands.

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METHODS AND MATERIALS

Insects and Gland Extracts. Bugs (all stages) were collected at regular intervals during 1990–1993 from an unsprayed citrus grove at the Maroochy Horticultural Research Station, Nambour, and from Cotton trees (*Hibiscus tiliaceus* L.) in the Maroochydore district. The collecting area is approximately 100 km north of Brisbane, Queensland. *P. plagipennis* was maintained in the laboratory on *Tenebrio molitor* L. larvae and pupae supplemented with moths from a light trap.

Adult *P. plagipennis* were killed by storage in a freezer. After thawing, male and female bugs were dissected under tap water. The abdominal cavity of each bug was opened up dorsally exposing the pheromone glands. All fat body surrounding the glands was carefully removed. Pheromone glands were excised, blotted with tissue paper, and placed individually in small conical vials containing approximately 250 μl of CH_2Cl_2. They were then macerated with a melting point capillary tube.

Airborne Trapping of Bug Volatiles. Active trapping of airborne compounds was achieved by placing single insects in an all-glass system comprising a cylinder of ca. 150 ml with charcoal pre- and post-filters. Air was aspirated at ca. 30 ml/min through the series-connected system comprised of an activated carbon prefilter, cylinder, and Swinney Luer-Lok filter holder packed with ca. 30 mg activated carbon. Sampling normally continued for 24 hr.

Trapped compounds were desorbed from the carbon by detaching the filter holder from the apparatus, attaching it to a Luer-Lok syringe and eluting with CH_2Cl_2. Passive adsorption was achieved using proprietary stainless steel adsorption tubes (Perkin Elmer) packed with ca. 200 mg 60–80 mesh Tenax-TA. Subsequent desorption was achieved thermally at 200°C using the automated Perkin Elmer ATD-50 instrument, interfaced to the gas chromatograph–mass spectrometer (GC-MS), with helium carrier gas.

Chemical Analysis. Gland extracts and solvent-desorbed solutions were chromatographed on a nonpolar capillary column (0.25 μm film, 0.25 mm ID, 30 m DB-5, J&W Scientific, Folsom, California) housed in a Hewlett Packard 5890 GC. Injection was split and the oven was programmed from 45°C (2 min delay) to 260°C at 15°C/min. The carrier gas was nitrogen. GC-MS analyses of solutions and thermally desorbed analytes were performed on a capillary column of identical specifications to that described above, housed in a Finigan 1020B instrument. Mass spectra were generated by a 70 eV electron impact. Injection was splitless and the carrier gas helium. Resolution of synthetic racemic ester and determination of the absolute configuration of the natural compound was achieved by chromatography on a CDX-B (J&W) column of identical dimensions to that described above and operated isothermally at 100°C.

Chemical Manipulations and Syntheses. Transesterification of the posterior DAG contents was achieved by adding 1 ml methanolic boron trifluoride (14%) to the gland extract. After standing overnight, 5 ml aqueous sodium bicarbonate and 1 ml hexane were added, the mixture shaken, and the hexane solution removed.

The racemic major ester was synthesized by azeotropic distillation of water from a benzene solution (150 ml) of 5 g (*Z*)-3-hexenol, 5.5 g (*RS*)-2-hydroxy-3-methylbutyric acid and 50 mg *p*-toluenesulfonic acid. After 3 h, the solution was cooled, extracted with aqueous sodium bicarbonate, dried, and reduced under vacuum. Synthesis of the *R* enantiomer employed the acid derived from oxidative deamination (Mori, 1976) of D-valine as follows. (The *S* enantiomer was synthesized in identical fashion from L-valine). A solution of 10 g sodium nitrite in 20 ml water was added dropwise to 10 g D-valine in 40 ml molar sulfuric acid, with the temperature maintained below 5°C. The solution was allowed to warm to room temperature and stirred overnight, its pH adjusted to 3 if necessary, and then concentrated under vacuum. (A lower pH, resulting from the use of more than an exact equivalent of sulfuric acid, was associated with significant isomerization at the double bond during subsequent esterification). The resultant semisolid was extracted into ether, the ether dried and concentrated, and the residue dissolved in chloroform and dried further. Evaporation of the solvent yielded 8.2 g (81%) acid, which was used without further purification. The ester (12 g, 85%) derived from this chiral acid was distilled under reduced pressure (120°C, 5 mm). The level of contaminating *E* isomer was generally less than 5%. The racemic acid, amino acid enantiomers, and alcohol used in the syntheses were Aldrich Chem. Co. reagents, as were the remaining components identified in gland extracts and included in artificial blends.

Bioactivity Studies: Forced Air Olfactometer. A Perspex Y-tube olfactometer was used to determine the response of mated female *P. plagipennis* to heptane extracts of male posterior dorsal abdominal glands (DAGs), live calling males, and various formulations of synthetic pheromone. Bugs could only walk in the olfactometer (10 mm diam., arms 22 cm long), and each arm ended in a rectangular plastic container (17 × 12 × 5 cm). Clean air (from outside the testing room) was passed through the containers via plastic tubing from an air pump (85–100 ml/min). The holding chamber for bugs was also a plastic container with a muslin-covered rear hole through which the air current exited the system. A smoke test demonstrated a laminar airflow in both arms and in the base tube. Bugs walked upwind in the base tube, chose an arm, and ended up in the sample or control chamber. Tests were conducted in a constant environment room (30.0 ± 0.5°C, 50 ± 10% relative humidity). Lighting was provided by two 40-W fluorescent tubes located in front of the two arms to encourage bug movement. Each test was conducted over a period of 6–8 hr and four to

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seven females were used per test. Records of movement were taken at 10-min intervals with bugs returned to the holding chamber.

Male DAG extracts were prepared by excising the posterior paired glands from a single bug under a microscope and placing them in a glass vial containing 1 ml of heptane. The glands were lightly crushed with a glass rod, and vials were covered with plastic film with a single pin hole. A vial with heptane only was used in the control chamber.

Live males, separated from females for at least three weeks, were presented in glass specimen tubes with muslin lids. A single male was presented at a time and the experiment commenced when calling behavior was observed.

Five formulations of synthetic pheromone were tested: (1) the racemate; (2) the natural enantiomer (*Z*)*R*, (3) the unnatural enantiomer *S* of the major male posterior DAG ester and two blends of the ester [(*Z*)*R* enantiomer] with minor components identified in airborne trappings and DAG extracts (4) 75% ester, 12.5% 2-phenylethanol, 12.5% 3-methylbutanol and (5) 60% ester, 15% 2-phenylethanol, 15% 3-methylbutanol, 5% (*E*)-2-hexenoic acid, 5% (*Z*)-3-hexenol). All synthetic materials were used at a rate of 100 mg/ml heptane in a glass vial covered with plastic film (punctured). Vials with heptane only served as controls.

Five to 19 replicates were performed for each material (synthetic samples, DAG extracts, live males) over a five-month period. Female *P. plagipennis* were "rested" for three to four days between each test to avoid habituation and were isolated from males during rest periods. To compensate for possible minor asymmetry in the olfactometer or experimental conditions, test and control samples were alternated between arms between each test. After each test, the olfactometer was washed thoroughly in hot water and acetone. Sporadic tests using heptane only in both chambers demonstrated neutrality of the system to female *P. plagipennis*. Data were subjected to chi-squared analysis.

Bioactivity Studies: Still-Air Olfactometer. A cage–funnel trap arrangement was used as a dual-choice still-air olfactometer. Two types of cage were used: (1 steel frame and meshed (60 × 30 × 30 cm) and (2 wooden framed, cloth meshed (60 × 45 × 45 cm). Funnel traps were constructed from Perspex cylinders (27 × 15 cm) with two holes (4 cm diam.) on opposite sides. Each hole was covered by an inwardly projecting screen funnel. Traps were baited with test material, control solutions (heptane only), or left unbaited. Bugs responded to traps by locomotion and/or flight. Tests were conducted at 27 or 30°C (±0.5°C) and ran for periods of 6–96 hr. Where tests ran for 24 hr or more, photophase was 15 hr. Most tests were conducted using 4–33 virgin or mated female *P. plagipennis* and the number of trapped bugs was recorded twice daily (0800 and 1600 hr). One series of tests used 13–17 mated males. Trapped bugs were reliberated in the cage. DAG extracts, calling males, and synthetic samples of the (*Z*)*R* enantiomer of the major male posterior DAG ester and two

blends of the ester with minor components were tested against females [80% blend: 80% ester, 10% (*Z*)-3-hexenol, 5% 2-phenylethanol, 2% decanal, 2% 3-methylbutanol, 1% (*E*)-2-hexenoic acid; 60% blend: 60% ester, 15% 2-phenylethanol, 15% 3-methylbutanol, 5% (*E*)-2-hexenoic acid, 5% (*Z*)-3-hexenol]. In addition, synthetic samples of the *R* enantiomer of the ester that were "contaminated" with 30–60% of the *E* isomer, were also tested. The (*Z*)-*R* enantiomer was also tested against males. DAG extracts were prepared and presented in glass vials as described previously. Single calling males were presented in glass specimen tubes with muslin lids. Synthetic materials were prepared at a rate of 25 mg/ml heptane and presented in uncovered glass vials. Three to seven replicates were conducted for each material. Female *P. plagipennis* were rested for at least three days between each test and were isolated from males. Food (moths, mealworms) was available for the bugs at all times during tests. Data were subjected to analysis of variance.

Bioactivity Studies: Flight Cage. Two outdoor flight cages (A: 3 × 3 × 1.8 m and B: 6.6 × 3 × 1.8 m) were used to test the response of mated female *P. plagipennis* to three synthetic materials: (1) (*Z*)*R* enantiomer of the major male posterior DAG ester, (2) 60% ester blend, and (3) 80% ester blend (blend formulations as described previously). The flight cages were constructed of a steel rod framework covered with green shadecloth. Cages were placed in an open sunlit grassy area.

Experiment 1 was conducted in flight cage A over 43 days (March 3–April 15). Six cylinder/funnel traps (same design as described previously) were hung from the central strut of the cage, spaced evenly from one side of the cage to the other. Two traps were baited with 25 mg of the (*Z*)*R* enantiomer in 1 ml of heptane, two with the 60% blend in 1 ml of heptane and two with heptane alone. All solutions were held in glass vials covered with punctured plastic film. Pheromones were replaced and traps repositioned weekly. Ten female *P. plagipennis* were released into the cage. Food for the bugs was available at all times with insects from a light trap released in the cage on alternate days. Traps were checked daily (1400–1500 hr). Trapped bugs were recorded and released back into the cage. Four females were still alive at the end of the experiment.

Experiment 2 was conducted in flight cage B during November 15–22, 1993. Four cylinder/funnel traps were attached to supporting stakes of potted citrus (Valencia orange) plants, 1.5 m tall. Eight plants were placed in a line from one end of the cage to the other (6.6 m) and traps attached to alternate plants. Two traps were baited with 25 mg of the *R* enantiomer in 1 ml of heptane, and two traps contained vials of heptane only. Traps with pheromones were separated by control traps. Pheromones were replaced at three-day intervals and traps were checked twice daily (0800 and 1400 hr). Twenty-five female *P. plagipennis* were released in the cage and retrieved at the conclusion of the experiment.

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Cage. Two outdoor flight cages (A: 3 × 3 × were used to test the response of mated female materials: (1) (*Z*)*R* enantiomer of the major % ester blend, and (3) 80% ester blend (blend usly). The flight cages were constructed of a n green shadecloth. Cages were placed in an

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RESULTS

Dissection of adult *P. plagipennis* verified the presence of three well-developed paired DAGs (Aldrich, 1991). There was marked sexual dimorphism with the anterior and posterior glands being much larger in males than females (Figure 1). The male posterior DAG was always larger (2–3×) than the anterior DAG and usually antlerlike. The mid-DAGs of both sexes were morphologically similar.

Gland Extracts. Chromatograms of the male posterior gland extracts were dominated (>90%) by a component whose mass spectrum failed to match any NIST data-base entry; *m/z* (%): 113(1), 99(9), 83(23), 82(100), 76(5), 73(43), 67(50), 55(24), 43(5), 41(10), 39(4). However, the presence of several abundant fragments consistent with a hexenyl group, together with the occurrence of (*Z*)-3-hexenol as a minor component in the chromatogram (confirmed by coinjection of authentic material) suggested a (*Z*)-3-hexenyl ester. Transesterification of the crude gland extract with boron trifluoride in methanol yielded increased quantities of the hexenol and a component whose mass spectrum closely matched (NIST data base) that of the methyl ester of 2-hydroxy-3-methylbutyric acid. Subsequent synthesis of the (*Z*)-3-hexenyl ester of this acid yielded a compound whose retention time (nonpolar column) and mass spectrum duplicated the nat-

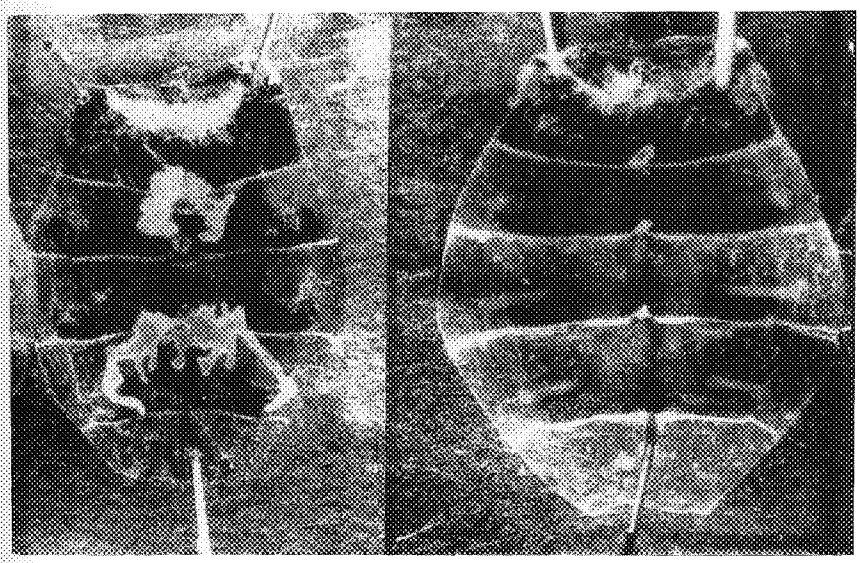

FIG. 1. Male (left) and female (right) *P. plagipennis* dissected to show anterior (upper), mid, and posterior dorsal abdominal glands.

ural compound exactly. Further characterization of the natural compound by gas chromatography on a chiral column established its absolute configuration as *R*, with an ee of >98%. Minor posterior gland components included decanal and three (*Z*)-3-hexenyl esters whose characterization is incomplete (Moore et al., 1993).

The male anterior DAG chromatographic profile was dominated by 3-methylbutanol (>75%), with 2-phenylethanol and (*E*)-2-hexenoic acid, all confirmed by coinjection of authentic standards.

The mid-DAG contents of both males and females were chromatographically identical and include at least 10 components. Chemical characterization is incomplete, but is considered irrelevant in the present context. The small size of the other female glands has so far precluded definitive study.

Airborne Volatiles Analysis. Chromatograms of airborne samples associated with males that appeared to be calling (deflexing abdomen and arching wings) trapped on carbon or Tenax displayed similar profiles. They were dominated by the major posterior DAG component (ca. 60%), but included the minor components present in this gland and the anterior DAG. No mid-DAG components were present. These profiles formed the basis for the artificial blends (Aldrich, 1991; Moore et al., 1993).

Bioactivity Studies: Forced-Air Olfactometer. Female *P. plagipennis* were significantly (<0.001) attracted to male posterior DAG extracts, calling males, the natural enantiomer *R* of the DAG major ester, and two blends containing the (*Z*)*R* enantiomer and associated minor components. There was no response

TABLE 1. ATTRACTION OF MATED FEMALE *P. plagipennis* TO VARIOUS NATURAL AND SYNTHETIC PHEROMONE SOURCES IN FORCED-AIR OLFACTOMETER TESTS

Test material	Replicates	Responses (%) to: Test material	Responses (%) to: Control	χ^2
Male posterior DAG extract	5	117[a] (69.6)	61	17.6
"Calling" Male	19	295[a] (74.7)	100	96.3
Racemate of male posterior DAG ester	12	91 (47.6)	100	0.42
Natural enantiomer (*R*) of male posterior DAG ester	17	273[a] (83.2)	55	144.9
Unnatural enantiomer (*S*) of male posterior DAG ester	17	142 (46.7)	162	1.3

[a] Significantly greater than control response ($P < 0.001$).

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EMALE *P. plagipennis* TO VARIOUS NATURAL AND
CES IN FORCED-AIR OLFACTOMETER TESTS

	Responses (%) to		
Replicates	Test material	Control	χ^2
5	117[a] (69.6)	61	17.6
19	295[a] (74.7)	100	96.3
12	91 (47.6)	100	0.42
17	273[a] (83.2)	55	144.9
17	142 (46.7)	162	1.3

e ($P < 0.001$).

to the racemate of the major ester or the unnatural enantiomer *S* (Table 1). The natural enantiomer (*Z*)*R* appeared to be the most attractive material tested with bugs responding in a ratio of almost 5 : 1.

Bioactivity Studies: Still-Air Olfactometer. Female *P. plagipennis* were significantly attracted ($P < 0.05$) to male posterior DAG extracts, calling males, the (*Z*)*R* enantiomer of the DAG major ester, and the two blends of the (*Z*)*R* enantiomer and minor components (Tables 2–3). Males were also significantly attracted to the (*Z*)*R* enantiomer (Table 4). There was no response to synthetic materials contaminated with the *E* isomer of *R* (Table 5). The response rate of females in these tests was fairly consistent at ca. 22–31%. Females tested three to seven days since last mating did not respond to the (*Z*)*R* enantiomer, but did so when retested at seven to ten days after mating (Table 6).

Bioactivity Studies: Flight cage. Both experiments demonstrated a clear response by female *P. plagipennis* to find and enter synthetic pheromone-baited traps (Table 7). Females appeared to respond equally well to (*Z*)*R* and the (*Z*)*R* blend.

TABLE 2. ATTRACTION OF VIRGIN AND MATED FEMALE *P. plagipennis* TO MALE POSTERIOR DAG EXTRACTS AND CALLING MALES IN STILL-AIR OLFACTOMETER TESTS

				Responses (*N*) to	
Replicate	Females (*N*) and mating status	Duration of test (hr)	Temperature (°C)	Male posterior DAG extract	Heptane (control)
1	4 mated	48	30	1	0
2	9 mated	20	30	2	0
3	8 mated	24	30	1	0
4	11 mated	24	30	1	0
5	11 mated	6	27	2	0
6	7 mated	34	30	1	0
Mean ± SE				1.3 ± 0.2[a]	0
				Calling male	Unbaited (control)
1	4 virgin	39	30	2	0
2	6 virgin	6	27	1	0
3	5 virgin	48	30	1	0
4	17 virgin	48	30	2	0
Mean ± SE				1.5 ± 0.25[a]	0

[a]Significantly more than control ($P < 0.05$).

TABLE 3. ATTRACTION OF VIRGIN AND MATED FEMALE *P. plagipennis* TO (*Z*)*R* ENANTIOMER OF MALE POSTERIOR DAG MAJOR ESTER AND BLENDS CONTAINING (*Z*)*R* AND ASSOCIATED MINOR COMPONENTS IN STILL-AIR OLFACTOMETER TESTS

Replicate	Females (*N*) and mating status	Duration of test (hr)	Temperature (°C)	Responses (*N*) to (*Z*)*R*	Responses (*N*) to Heptane (control)
1	11 virgin	48	27	2	0
2	11 virgin	31	27	2	0
3	20 virgin	32	30	4	0
4	18 virgin	54	30	3	1
5	18 virgin	31	30	6	0
6	18 mated	96	30	4	0
7	14 mated	96	30	4	1
Mean ± SE				3.6 ± 0.5[a]	0.25 ± 0.2
				(*Z*)*R* blend	Heptane (control)
1	12 mated	48	30	3[b]	0
2	21 virgin	48	30	3[c]	0
3	8 virgin	54	30	4[c]	1
4	10 virgin	31	30	6[c]	1
Mean ± SE				4 ± 0.6[a]	0.5 ± 0.25

[a] Significantly more than control ($P < 0.05$).
[b] 60% blend.
[c] 80% blend (see text).

TABLE 4. ATTRACTION OF MATED MALE *P. plagipennis* TO (*Z*)*R* ENANTIOMER OF MALE POSTERIOR DAG MAJOR ESTER

Replicate	Males (*N*)	Duration of test (hr)	Temp (°C)	Responses (*N*) to (*Z*)*R*	Responses (*N*) to Heptane (control)
1	13	96	30	5	1
2	17	96	30	1	0
3	17	96	30	4	1
Mean ± SE				3.3 ± 1.0[a]	0.7 ± 0.3

[a] Significantly more than control ($P < 0.05$).

MATED FEMALE *P. plagipennis* TO (*Z*)*R*
AJOR ESTER AND BLENDS CONTAINING (*Z*)*R*
TS IN STILL-AIR OLFACTOMETER TESTS

of	Temperature (°C)	Responses (*N*) to (*Z*)*R*	Responses (*N*) to Heptane (control)
	27	2	0
	27	2	0
	30	4	0
	30	3	1
	30	6	0
	30	4	0
	30	4	1
		3.6 ± 0.5[a]	0.25 ± 0.2
		(*Z*)*R* blend	Heptane (control)
	30	3[b]	0
	30	3[c]	0
	30	4[c]	1
	30	6[c]	1
		4 ± 0.6[a]	0.5 ± 0.25

P. plagipennis TO (*Z*)*R* ENANTIOMER OF
DAG MAJOR ESTER

Temp (°C)	Responses (*N*) to (*Z*)*R*	Responses (*N*) to Heptane (control)
30	5	1
30	1	0
30	4	1
	3.3 ± 1.0[a]	0.7 ± 0.3

TABLE 5. RESPONSES OF VIRGIN AND MATED FEMALE *P. plagipennis* TO *E* ISOMER CONTAMINATED (30–60%) (*Z*)*R* ENANTIOMER OF MALE POSTERIOR DAG MAJOR ESTER AND 60% BLEND CONTAINING (*Z*)(*E*)*R* AND ASSOCIATED MINOR COMPONENTS IN STILL-AIR OLFACTOMETER TESTS

Replicate	Females (*N*) and mating status	Duration of test (hr)	Temperature (°C)	Responses (*N*) to (*Z*)(*E*)*R*	Responses (*N*) to Heptane (control)
1	12 virgin	48	30	0	0
2	7 virgin	96	27	0	0
3	7 virgin	96	27	1	0
4	10 virgin	96	30	0	1
5	13 virgin	48	27	0	0
Mean ± SE				0.2 ± 0.2[a]	0.2 ± 0.2
				60% (*Z*)(*E*)*R* blend	Heptane (control)
1	12 virgin	96	30	0	0
2	21 virgin	96	30	0	1
3	33 virgin	96	30	1	0
4	15 virgin	96	30	0	1
5	11 virgin	96	30	1	0
6	14 virgin	96	30	1	1
Mean ± SE				0.5 ± 0.2[a]	0.5 ± 0.2

[a]No significant difference from control ($P > 0.05$).

DISCUSSION

This study presents the first evidence of an aggregation pheromone produced by a reduviid. Male *P. plagipennis* possess three pairs of DAGs, of which the anterior and posterior pair are considerably larger than the corresponding glands in females (Aldrich, 1991). Calling males release a pleasant-smelling odor from these glands comprised largely (ca. 60%) of a novel ester, (*Z*)-3-hexenyl (*R*)-2-hydroxy-3-methylbutyrate, with 3-methylbutanol, 2-phenylethanol, (*Z*)-3-hexenol, decanal, (*E*)-2-hexenoic acid, and three minor hexenyl esters, as minor components. The major ester alone, or in combination with minor components, was attractive to female *P. plagipennis* in olfactometer and flight-cage tests. The major ester was also attractive to males, indicating that the compound functions as an aggregation pheromone. It is possible that addition of one or more of the minor hexenyl esters might increase potency of the pheromone and this should be studied further.

TABLE 6. EFFECT OF TIMING OF LAST MATING ON RESPONSIVENESS OF FEMALE *P. plagipennis* TO (*Z*)*R* ENANTIOMER OF MALE POSTERIOR DAG MAJOR ESTER IN STILL-AIR OLFACTOMETER TESTS (SAME FEMALES USED IN BOTH SERIES)

Replicate	Females (*N*)	Duration of test (hr)	Temperature (°C)	Responses (*N*) to (*Z*)*R*	Responses (*N*) to Heptane (control)
3–7 days since mating					
1	16	48	30	0	1
2	10	48	27	0	0
3	10	48	27	0	0
Mean ± SE				0	0.3 ± 0.3
7–10 days since mating					
1	16	48	30	2	0
2	10	48	27	3	0
3	10	48	27	3	0
Mean ± SE				2.7 ± 0.3[a]	0

[a]Significantly greater than control ($P < 0.05$).

TABLE 7. NUMBER OF FEMALE *P. plagipennis* TRAPPED IN PHEROMONE-BAITED AND UNBAITED TRAPS IN TWO FLIGHT-CAGE EXPERIMENTS

	(*Z*)*R*	60% (*Z*)*R* blend	Unbaited
Experiment 1, March 3–April 15, 1993 (10 females)	5	5	0
Experiment 2, November 15–22, 1993 (25 females)	5		0

Only the natural *R* enantiomer of the major ester was attractive. Nonattractivity of the racemate indicates the *S* enantiomer inhibits the action of the antipode. Inhibition of the biological activity of one enantiomer by its antipode has been noted before (Tumlinson et al., 1977), but a situation where a synthetic racemate successfully mimics a chiral pheromone because of the biological inactivity of the antipode is more common (Mori, 1989).

The presence of 30–60% of the *E* isomer in samples of the major ester rendered the compound unattractive to *P. plagipennis*. Progressive environmental isomerization of the pheromone of the pea moth (*Cydia nigricana* F.) was reported to inhibit attraction of this insect (Witzgall et al., 1993). Preliminary tests indicate the pheromone of *P. plagipennis* does not undergo rapid isomer-

LAST MATING ON RESPONSIVENESS OF FEMALE *P.*
MER OF MALE POSTERIOR DAG MAJOR ESTER
TESTS (SAME FEMALES USED IN BOTH SERIES)

ation of st (hr)	Temperature (°C)	Responses (*N*) to (*Z*)*R*	Heptane (control)
48	30	0	1
48	27	0	0
48	27	0	0
		0	0.3 ± 0.3
48	30	2	0
48	27	3	0
48	27	3	0
		2.7 ± 0.3[a]	0

0.05).

plagipennis TRAPPED IN PHEROMONE-BAITED AND
N TWO FLIGHT-CAGE EXPERIMENTS

	(*Z*)*R*	60% (*Z*)*R* blend	Unbaited
	5	5	0
	5		0

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es the *S* enantiomer inhibits the action of the
ical activity of one enantiomer by its antipode
et al., 1977), but a situation where a synthetic
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his insect (Witzgall et al., 1993). Preliminary
P. plagipennis does not undergo rapid isomer-

ization when exposed to field or laboratory conditions. However, it is clear that excessive isomeric contamination should be avoided during synthesis of the compound. It is possible that the minor (<5%) *E* isomer contamination that was present in the bioactive samples of (*Z*)*R* in this study may have limited the responses shown by *P. plagipennis*. A totally pure formulation, although difficult to achieve, should also be tested. Mating of *P. plagipennis* appeared to be followed by a period of about seven days when females did not respond to pheromone. It is also quite likely that responses to the pheromone vary with physiological age. Most tests in this study were conducted using young, virgin females, which are likely to show greatest response to an aggregation pheromone.

No attempt was made in this study to determine the effect of pheromone dosage on attraction of female *P. plagipennis*. Doses used were high (100 and 25 mg) and may have been excessive. Responses by females to male posterior DAG extracts were comparable to those for synthetic baits, despite, presumably, considerably lower doses of released pheromone. Excessive pheromone doses can cause adaptation, habituation, or confusion in target insects (Bartell, 1982). The effect of lower doses of pheromone on attraction of *P. plagipennis* should be evaluated.

An effective, synthetic aggregation pheromone for *P. plagipennis* would be of considerable value to more effective and widespread utilization of this predator in managing populations of the citrus stink bug pest *B. bibax*. Pheromone could be used to concentrate *P. plagipennis* populations in citrus orchards to enhance biological control. Pheromone could also be used to remove *P. plagipennis* from orchards prior to application of insecticides. Pheromone-baited traps may provide the ability to mass-trap *P. plagipennis* for release at sites or in regions in which it currently does not occur. The value of *P. plagipennis* in citrus orchards is not confined to regulating *B. bibax*. As a general predator, it also preys on other citrus pests such as caterpillars, mealybugs, weevils, etc. (James, 1994). Other perennial horticultural ecosystems in Queensland and northern New South Wales also benefit from the presence of *P. plagipennis*. In addition to being a tool for harnessing the biological control potential of *P. plagipennis* in horticultural crops, synthetic pheromone would also serve as a powerful research tool, enabling field evaluation of the role of this predator in biological control of specific pests. Assassin bugs as biological control agents have received even less attention than other general predators (Schaefer, 1988), largely due to the difficulties involved in evaluation (Luck et al., 1988).

Pheromonal manipulation of predatory heteropterans is the basis of HOPE (husbandry of pest enemies), a concept presented by Aldrich (1991). The first commercially available product for HOPE is the Spined Soldier Bug Attractor, based on the male-produced aggregation pheromone of this important North American predatory pentatomid (*Podisus maculiventris* Say) (Aldrich et al.,

1984). Aimed at the home garden market, the pheromone attracts *P. maculiventris* to aid in garden pest control. A similar market exists in Australia for a *P. plagipennis* attractant.

Much research remains before the use of pheromones in husbandry of *P. plagipennis* can be practiced. Rigorous field evaluations of dose and blends are required to ensure an optimal formulation is produced. More information is also needed on the role of aggregation pheromone in *P. plagipennis* biology to ensure appropriate use of and expectations from synthetic material.

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REFERENCES

ALDRICH, J.R., 1988. Chemical ecology of the Heteroptera. *Annu. Rev. Entomol.* 33:211–238.

ALDRICH, J.R. 1991. Pheromones of good and bad bugs. *News Bull. Entomol. Soc. Qld.* 19:19–27.

ALDRICH, J.R., KOCHANSKY, J.R., and ABRAMS, C.B. 1984. Attractant for a beneficial insect and its parasitoids: Pheromone of the predatory spined soldier bug, *Podisus maculiventris* (Hemiptera: Pentatomidae). *Environ. Entomol.* 13:1031–1036.

BARTELL, R.J. 1982. Mechanisms of communication disruption by pheromone in the control of a Lepidoptera: A review. *Physiol. Entomol.* 7:353–364.

BLUM, M.S. 1981. Chemical Defenses of Arthropods. New York, Academic Press, 562 pp.

JAMES, D.G. 1989. Population biology of *Biprorulus bibax* Breddin (Hemiptera: Pentatomidae) in a southern New South Wales citrus orchard. *J. Aust. Entomol. Soc.* 28:279–286.

JAMES, D.G. 1992. Effect of temperature on development and survivorship of *Pristhesancus plagipennis* (Hemiptera: Reduviidae). *Entomophaga* 37:259–264.

JAMES, D.G. 1993. The development of suppression tactics for *Biprorulus bibax* (Heteroptera: Pentatomidae) as part of an integrated pest management program in inland citrus of southeastern Australia. *Bull. Entomol. Res.* 84:31–38.

JAMES, D.G. 1994. Prey consumption by *Pristhesancus plagipennis* Walker (Hemiptera: Pentatomidae) during development. *Aust. Entomol.* 21:43–47.

JAMES, D.G., and WARREN, G.N. 1989. Sexual dimorphism of dorsal abdominal glands in *Biprorulus bibax* Breddin (Hemiptera: Pentatomidae). *J. Aust. Entomol. Soc.* 28:75–76.

JAMES, D.G., FAULDER, R.J., and WARREN, G.N. 1990. Phenology of reproductive status, weight and lipid reserves of *Biprorulus bibax* (Hemiptera: Pentatomidae). *Environ. Entomol.* 19:1710–1715.

LUCK, R.F., SHEPARD, B.M., and KENMORE, P.E. 1988. Experimental methods for evaluating arthropod natural enemies. *Annu. Rev. Entomol.* 33:367–391.

MOORE, C.J., ALDRICH, J.R., and JAMES, D.G. 1993. Some aspects of the scent gland chemistry of the assassin bug, *Pristhesancus plagipennis* Walker (Hemiptera: Reduviidae), pp. 304–306, *in* S.A. Corey, K.J. Dall, and W.M. Milne (eds.). Pest Control and Sustainable Agriculture. CSIRO Publications, Canberra, Australia.

MORI, K. 1976. Synthesis of optically active forms of ipsenol, the pheromone of *Ips* bark beetles. *Tetrahedron* 32:1101–1106.

rket, the pheromone attracts *P. maculi-*
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RENCES

Heteroptera. *Annu. Rev. Entomol.* 33:211–238.
bad bugs. *News Bull. Entomol. Soc. Qld.* 19:19–

C.B. 1984. Attractant for a beneficial insect and
spined soldier bug, *Podisus maculiventris* (Hem-
3:1031–1036.
ation disruption by pheromone in the control of a
7:353–364.
pods. New York, Academic Press, 562 pp.
rulus bibax Breddin (Hemiptera: Pentatomidae) in
J. Aust. Entomol. Soc. 28:279–286.
velopment and survivorship of *Pristhesancus pla-*
haga 37:259–264.
ession tactics for *Biprorulus bibax* (Heteroptera:
management program in inland citrus of south-
1–38.
sancus plagipennis Walker (Hemiptera: Pentatom-
1:43–47.
dimorphism of dorsal abdominal glands in *Bipro-*
dae). *J. Aust. Entomol. Soc.* 28:75–76.
N. 1990. Phenology of reproductive status, weight
ptera: Pentatomidae). *Environ. Entomol.* 19:1710–

P.E. 1988. Experimental methods for evaluating
mol. 33:367–391.
1993. Some aspects of the scent gland chemistry
is Walker (Hemiptera: Reduviidae), pp. 304–306,
(eds.). Pest Control and Sustainable Agriculture.

ms of ipsenol, the pheromone of *Ips* bark beetles.

MORI, K. 1989. Synthesis of optically active pheromones. *Tetrahedron* 45:3233–3298.

MURRAY, B. (ed.). 1987. Protect Your Citrus. Department of Primary Industries, Brisbane, Queensland. Information Series: Q18701.

SCHAEFER, C.W. 1988. Reduviidae (Hemiptera: Heteroptera) as agents of biological control. *Biocovas* 1:27–33.

STADDON, B.W. 1979. The scent glands of Heteroptera. *Adv. Insect Physiol.* 14:351–418.

SUMMERVILLE, W.A.T. 1931. The larger horned citrus bug. Queensland Department of Agriculture and Stock, Division of Entomology and Plant Pathology Bulletin No. 8.

TUMLINSON, J.H., KLEIN, M.G., DOOLITTLE, R.E., LADD, T.L., and PROVEAUX, A.T. 1977. Identification of the female Japanese beetle sex pheromone: Inhibition of male response by an enantiomer. *Science* 197:789–792.

WITZGALL, P., BENGTSSON, M., UNELIUS, C.R., and LÖFQVIST, J. 1993. Attraction of pea moth, *Cydia nigricana* F. (Lepidoptera: Tortricidae) to female sex pheromone (*E,E*)-8,10-dodecadien-1-yl acetate, is inhibited by geometric isomers *E,Z*, *Z,E*, and *Z,Z*. *J. Chem. Ecol.* 19:1917–1927.